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Article

Integrin Subunit CD18 Is the T-Lymphocyte Receptor for the *Helicobacter pylori* Vacuolating Cytotoxin

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SUMMARY

Helicobacter pylori **infection is associated with gastritis, ulcerations, and gastric adenocarcinoma. *H. pylori* secretes the vacuolating cytotoxin (VacA), a major pathogenicity factor. VacA has immunosuppressive effects, inhibiting interleukin-2 (IL-2) secretion by interference with the T cell receptor/IL-2 signaling pathway at the level of calcineurin, the Ca^{2+}-calmodulin-dependent phosphatase. Here, we show that VacA efficiently enters activated, migrating primary human T lymphocytes by binding to the β2 (CD18) integrin receptor subunit and exploiting the recycling of lymphocyte function-associated antigen (LFA)-1. LFA-1-deficient Jurkat T cells were resistant to vacuolation and IL-2 modulation, and genetic complementation restored sensitivity to VacA. VacA targeted human, but not murine, CD18 for cell entry, consistent with the species-specific adaptation of *H. pylori*. Furthermore, expression of human integrin receptors (LFA-1 or Mac-1) in murine T cells resulted in VacA-mediated cellular vacuolation. Thus, *H. pylori* co-opts CD18 as a VacA receptor on human T lymphocytes to subvert the host immune response.**

INTRODUCTION

Helicobacter pylori (*Hp*) persistently infects the gastric mucosa of more than half the world's population. *Hp* causes peptic ulcer disease and is an early risk factor for gastric cancer (Suerbaum and Michetti, 2002). The bacterium is equipped with a set of remarkable pathogenicity factors, including up to three distinct type IV secretion systems (T4SS) and the vacuolating cytotoxin (VacA). The *cag*-pathogenicity island (*cag*-PAI) encodes a T4SS involved in translocation of the cytotoxin-associated antigen (CagA) into host cells (Odenbreit et al., 2000). Furthermore, *cag*-PAI positive strains are more frequently associated with severe gastric inflammation, ulceration, and an increased risk to develop gastric cancer (Blaser et al., 1995).

The vacuolating cytotoxin induces cellular vacuolation in epithelial cells (Cover and Blaser, 1992). VacA is produced as a 140 kDa precursor protein and actively secreted from *Hp* by a type V autotransporter mechanism (Fischer et al., 2001). The 95 kDa secreted VacA protein varies in the signal sequence (s1a, s1b, s1c, and s2) and/or its middle region (m1 and m2) between different *Hp* strains (Atherton et al., 1995). In contact with host cells, the extracellular 95 kDa acid-activated VacA forms anion-selective channels by oligomerization of membrane-bound monomers (Czajkowsky et al., 1999) and is subsequently internalized into eukaryotic cells (reviewed in Cover and Blanke, 2005). Vacuole biogenesis requires the activity of the Rab7 GTPase and the V-type ATPase. VacA treatment of epithelial monolayers in vitro also causes reduction in transepithelial resistance (Papini et al., 1998).

Several lines of evidence indicate that the initial interaction of VacA with its target cells is through (a) high affinity receptor(s) (Massari et al., 1998), which facilitates its interaction with the cell membrane to form the channels, which are subsequently internalized. Kinetic and inhibition analyses suggested the presence of at least two receptors on epithelial cells, an m1-type specific high-affinity receptor and a common VacA receptor interacting similarly with m1 and m2 VacA via a lower affinity (Wang et al., 2001). Three cell surface proteins have been implicated so far as specific receptors for VacA. These include the epidermal growth factor receptor (EGFR) (Seto et al., 1998) as well as receptor-like tyrosine phosphatase (RPTP) α (Yahiro et al., 2003) and RPTPβ (Yahiro et al., 1999; Fujikawa et al., 2003).

Upon contact with T lymphocytes, VacA is able to inhibit cell proliferation and to interfere with the T cell receptor/interleukin-2 (IL-2) signaling pathway at the level of the Ca^{2+}-calmodulin-dependent phosphatase calcineurin (Gebert et al., 2003; Boncristiano et al., 2003; Sundrud et al., 2004). Nuclear translocation of Nuclear Factor of Activated T cells (NFAT), a transcription factor acting as a global regulator of immune response genes, is abrogated, resulting in downregulation of IL-2 transcription (Gebert et al., 2003; Boncristiano et al., 2003). Thus, it has been suggested that VacA partially mimics the activity of the immunosuppressive drugs cyclosporine A or FK506 by inducing a local immune suppression, explaining the extraordinary chronicity of *Hp* infections. Despite this rather specific interference of VacA

with the T cell activation pathway, the molecular interactions that would explain the VacA specificity for T cells or for leucocytes, in general, were unclear.

In the present study, we identified the β2 (CD18) integrin subunit as a leucocyte-specific receptor for m1-VacA on human T cells. As a heterodimeric transmembrane receptor, CD18 may combine with different integrin α subunits, such as CD11a (to produce LFA-1), CD11b (to generate Mac-1, also known as complement receptor 3, CR3), CD11c, or CD11d. An essential role of CD18 as a VacA receptor was convincingly demonstrated by the use of CD18-deficient human T cells and by expression of different combinations of human CD18 integrin heterodimers in chinese hamster ovary (CHO) cells. A direct interaction of m1-VacA and CD18 was evident from immunoprecipitation experiments. We also show that murine T cells are not adequately targeted by m1-VacA, which has major implications for the widely used *Hp* mouse model.

RESULTS

Inducible VacA Sensitivity of Primary Human CD4$^+$ T Cells

Earlier studies showed that VacA treatment of a T cell line (Jurkat E6.1) strongly reduced IL-2 gene transcription and IL-2 secretion (Gebert et al., 2003; Boncristiano et al., 2003; Sundrud et al., 2004). However, VacA did not significantly alter IL-2 secretion of human CD4$^+$ T cells, while it diminished their proliferation (Sundrud et al., 2004). We now observe that pretreatment of primary human CD4$^+$ T cells with phorbol myristate acetate (PMA) (30 min) before adding *Hp* P12 bacterial supernatant (m1-VacA) or purified VacA strongly increased the sensitivity of these cells toward VacA (Figure 1A, columns CD4/PMA). A similar albeit lower VacA sensitivity of human CD4$^+$ T cells was inducible by their preactivation with α-CD3/CD28-coated polystyrene beads (hereafter termed α-CD3/CD28-PSB) (see Figure S1 available with this article online), but not when soluble α-CD3/CD28 antibodies were used concomitantly with VacA (Figure 1A). Resting CD4$^+$ T cells not preactivated by PMA or α-CD3/CD28-PSB did not respond to VacA-mediated inhibition of IL-2 secretion (Figure 1A).

To study the binding and internalization of VacA into primary human CD4$^+$ T cells, VacA was purified from *Hp* culture supernatant (Cover et al., 1997) and labeled with the Alexa 488/555 dye ($VacA_{Alexa}$). Live Confocal Laser Scanning microscopy revealed that Jurkat T cells bound acid-activated $VacA_{Alexa}$ on the cell surface and internalized it independently from any prior PMA activation of the cells (Figure 1B). In contrast, resting CD4$^+$ T cells also interacted with acid-activated VacA on the surface but showed only minimal or no VacA internalization (Figure 1C). However, a 30 min pretreatment of CD4$^+$ T cells with PMA resulted in binding of $VacA_{Alexa}$ on the cellular surface, as well as its significant internalization into the CD4$^+$ T cells (Figure 1C). Treatment of PMA-activated CD4$^+$ T cells with calpeptin, a specific inhibitor of the Ca^{2+}-dependent cellular protease calpain, abolished uptake of $VacA_{Alexa}$ (Figure 1C). Quantification of m1-VacA uptake based on quenching of surface associated $Alexa_{488}$ fluorophore revealed a significant VacA internalization in PMA-treated versus nontreated CD4$^+$ T cells (Figure 1D). Thus, the polyclonal activation of resting human CD4$^+$ T cells with PMA primes these cells to

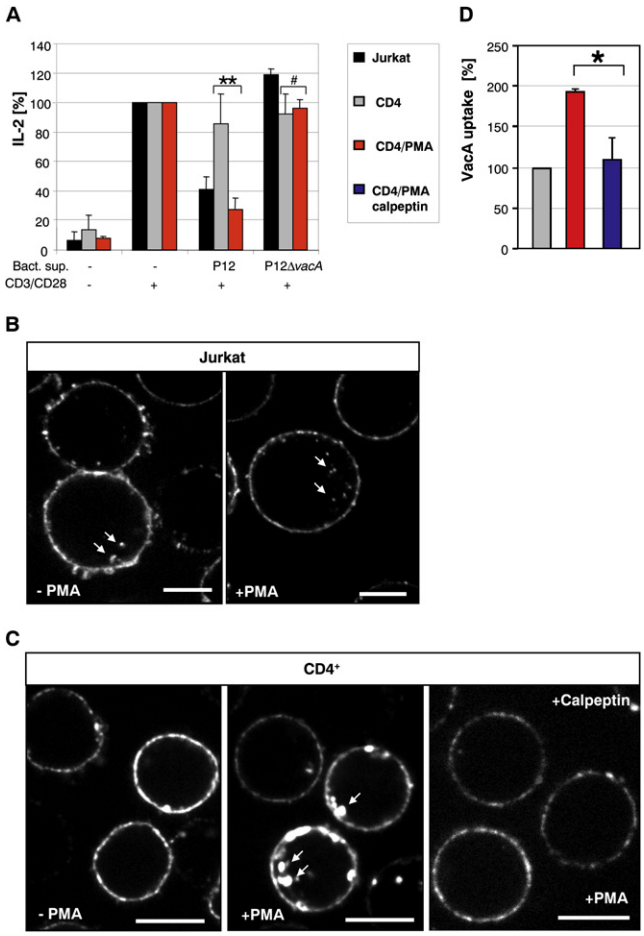

Figure 1. VacA Sensitivity of Primary Human CD4$^+$ T Cells

(A) Sandwich enzyme-linked immunosorbent assay (ELISA) to measure secreted IL-2. Jurkat T cells and primary human CD4$^+$ T cells ± PMA pretreatment were incubated with concentrated culture supernatant (ccs) of a *Hp* WT strain (P12) or VacA-negative mutant strain (P12Δ*vacA*) and activated with α-CD3/CD28-PSB as indicated. Data points represent the mean ± SEM from five experiments.

(B and C) m1-$VacA_{Alexa}$ (seen as white label) was incubated with Jurkat (B) or human CD4$^+$ T cells (C). For activation, T cells were treated with PMA only. Eventually, cells were treated with the calpain inhibitor calpeptin, as indicated.

(D) Quantification of $VacA_{Alexa}$ into CD4$^+$ T cells using a fluorescence quenching technique (see "Quantification of VacA Internalization" in the Supplemental Data). VacA uptake in nonstimulated CD4$^+$ T cells was set to 100%. Data points represent the mean ± SEM from four experiments. Student's t test, normally distributed, two-tailed. **$p < 0.01$, *$p < 0.05$, #not significant. Bars in micrographs represent 5 μm.

VacA uptake, resulting in cellular vacuolation and inhibition of IL-2 signaling.

Human Migrating Primary CD4$^+$ T Cells Show a High and Specific VacA Binding Affinity

T cells are usually highly motile in lymph node and tissue, as can be visualized directly in a living lymph node using intravital two-photon microscopy (Miller et al., 2003). Integrins such as αLβ2 (LFA-1), interacting with intercellular adhesion molecule 1 or 2 (ICAM-1, ICAM-2) play an important role in T lymphocyte migration. Active migration of T cells mediated by the interaction of

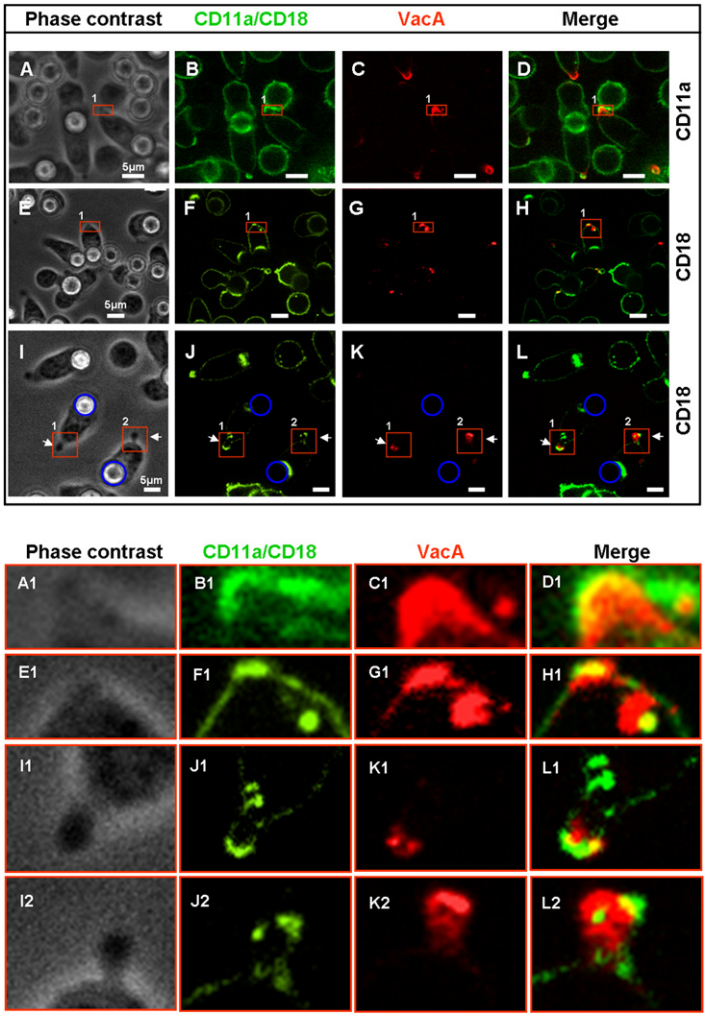

Figure 2. Colocalization of VacA with LFA-1 at the Uropod of Primary Human Migrating T Lymphocytes

Confocal fluorescence micrographs, demonstrating ubiquitous binding of $VacA_{Alexa}$ at the cell surface and its eventual uptake into cellular vesicles (arrows). (A–L) Human $CD4^+$ T cells activated by CD3/CD28-PSB on human ICAM-1-coated surface. Localization of CD11a or CD18 integrin subunits on T cells was determined by a CD11a-specific mAB (YTH81.5, AbD serotec) or CD18-specific mAB (IB4, Ancell) (green), as indicated. VacA was directly labeled with Alexa 555 ($VacA_{Alexa}$, red). The location of CD3/CD28-PSB on T cells is indicated by blue circles (I–L). The lower panel shows high magnifications of areas marked by red boxes (A1–L2) detailing distinct VacA or integrin localizations and their possible colocalization.

active LFA-1 with immobilized ICAM-1 can be induced in vitro by T cell receptor (TCR) crosslinking or PMA stimulation (Smith et al., 2003).

Following interaction with ICAM-1, activated T cells get polarized, attach at the leading edge, become motile, and migrate randomly (Hogg et al., 2004). In a mixed population of migrating and nonmigrating human $CD4^+$ T cells, resting T cells were labeled by $VacA_{Alexa}$ weakly but uniformly, whereas migrating T cells (flat and extended shape) were labeled more strongly and focally (Figure S2 and Movie S1). A high local enrichment of VacA was visible at the trailing edge (uropod) of polarized T cells (Figure S2 and Movie S2).

In many cell types, migration involves regulated endocytosis and exocytosis of integrins (Hogg et al., 2004). In T cells and primary neutrophils, unengaged LFA-1 is internalized and rapidly recycled upon chemoattractant stimulation via a clathrin-independent, cholesterol-sensitive pathway (Fabbri et al., 2005). Such a recycling may also be induced by PMA-mediated activation of PKC, which activates Rap1, a Ras-related GTPase modulating LFA-1 adhesive activity in T cells (Tohyama et al., 2003). Integrin internalization involves dynamic partitioning of integrin molecules into detergent-resistant membranes, the lipid rafts. In migrating T cells, lipid rafts are concentrated at the uropod. A preferential lipid raft association has also been described for VacA and its interaction with HeLa cells, which was essential for VacA cytotoxicity (Schraw et al., 2002). Blocking of calpain by the specific peptide inhibitor calpeptin, which interferes with integrin clustering (Hogg et al., 2003), abolished the uptake of VacA into PMA-activated $CD4^+$ T cells (Figures 1C and 1D). All these observations supported the hypothesis that m1-VacA might exploit integrin recycling vesicles in migrating T cells for its cellular uptake.

$VacA_{Alexa}$ Shows Colocalization with LFA-1

We first investigated whether LFA-1 might be involved in binding or uptake of VacA into T cells. Colocalization experiments were performed with CD3/CD28-PSB-activated primary human $CD4^+$ T cells and analyzed by live microscopy using a confocal spinning disc microscope (Perkin Elmer). Activated $CD4^+$ T cells (flat and extended shape) were visualized in the phase contrast image (Figures 2A, 2E, and 2I), with Alexa 488-labeled antibodies directed against integrin subunits CD11a or CD18 (green) (Figures 2B, 2F, and 2J) and Alexa 555-labeled VacA (red) (Figures 2C, 2G, and 2K). Most of the CD11a or CD18 label was concentrated either at the uropod, or in the clustered T cell receptor/CD3/CD28-PSB interaction region at the leading edge (Figures 2B, 2F, and 2J).

Interestingly, in polarized cells, VacA preferentially localized at the uropod (Figures 2C, 2G, and 2K and magnifications thereof in Figures 2C1, 2G1, and 2K1). Although partially distinct $VacA_{Alexa}$ and LFA-1 localization patterns were found, defined and reproducible colocalization of $VacA_{Alexa}$ and CD11a or CD18 was clearly obvious at the uropod (Figures 2D, 2H, and 2L and magnifications thereof in Figures 2D1, 2H1, 2L1, and 2L2). After 1 hr, VacA was frequently found together with LFA1 in small moving vesicles (see Figure S3A and Movie S3), whereas an intracellular perinuclear localization of $VacA_{Alexa}$ within $CD4^+$ T cells was routinely found after 12–16 hr (Figure S3B).

LFA-1-Deficient Jurkat T Cells Are Nonresponsive to VacA

The T cell line J-β2.7, derived from Jurkat E6.1 cells by chemical mutagenesis, is defective in CD11a production (Weber et al., 1997). Thus, J-β2.7 cells lack cell surface LFA-1 (Figure S4A). As compared to Jurkat E6.1, m1-VacA-mediated vacuolation of LFA-1-deficient J-β2.7 cells was reduced in a quantitative

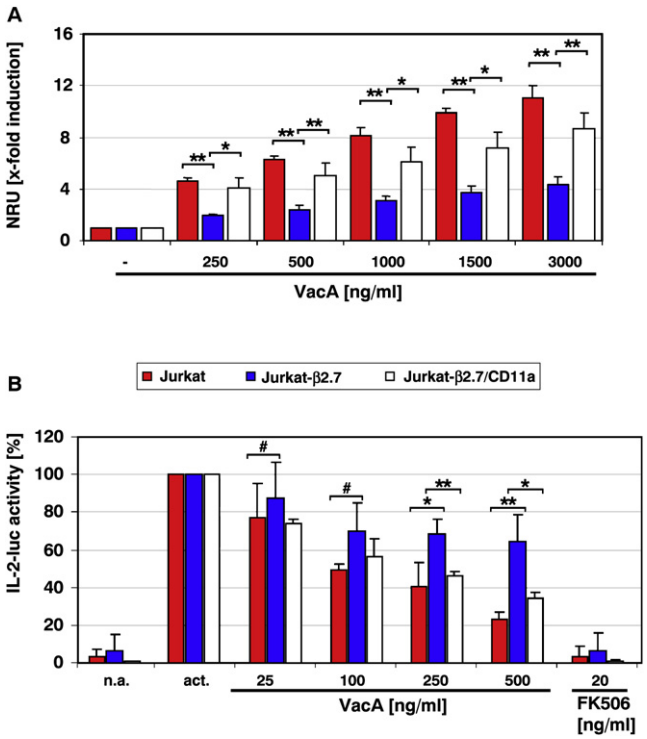

Figure 3. VacA Sensitivity of Jurkat T Cells Is Dependent on Functional CD18 Integrin on Cell Surface

(A) VacA-induced cellular vacuolation in Jurkat WT cells, Jurkat-β2.7 cells defective in CD18 integrin surface localization, or CD11a gene complemented Jurkat-β2.7 cells was measured by the quantitative neutral red assay. Vacuolation is presented as ×-fold induction compared to nontreated cells.

(B) Inhibition of IL-2 secretion in Jurkat T cells is dependent on CD18 integrin. A reporter plasmid carrying the complete IL-2 promoter coupled to the *luc* reporter gene (Gebert et al., 2003) was transfected into different Jurkat T cell lines (WT, Jurkat-β2.7 defective in LFA-1 surface expression, or CD11a gene complemented Jurkat-β2.7) and activated with PMA/ionomycin. The Luc activity of activated nontreated control was set to 100%. Cells were treated with purified VacA in increasing concentrations, as indicated. Student's t test, normally distributed, two-tailed. Data points represent the mean ± SEM from three experiments. $^{**}p < 0.01$, $^{*}p < 0.05$, $^{\#}$not significant. NRU, neutral red uptake; n.a., not activated; act, activated.

neutral red vacuolation assay (Cover et al., 1992), whereas functional complementation of Jurkat-β2.7 cells by transfection with CD11a (Jurkat-β2.7/CD11a) resulted in recovery of the vacuolating sensitivity (Figure 3A). A purified s1/m2-VacA (*H. pylori* strain P76) behaved similar to m1-VacA in vacuolation induction (Figure S5), suggesting that in Jurkat T cells this particular m2-VacA also seems to exploit the CD18 pathway. Since different genotypes of m2-VacA exist (m2a and m2b) (Ji et al., 2002), a general designation of a possible m2-VacA receptor awaits a further detailed analysis of additional m2-specific cytotoxins. To study the effect of VacA on calcineurin and IL-2 gene expression in a LFA-1-depleted cell line, Jurkat and J-β2.7 cells were transiently transfected with a pcDNA3-based luciferase (*luc*) reporter construct carrying the IL-2 gene promoter (IL-2-*luc*) (Gebert et al., 2003). The cells were treated with increasing concentrations of purified, acid-activated m1-VacA.

In the PMA/ionomycin-activated Jurkat cell line, VacA inhibited IL-2-*luc* gene transcription already at very low concentrations (100 ng/ml) in a concentration-dependent manner (Figure 3B). The LFA-1-deficient J-β2.7 T cells showed only a minimal reduction in IL-2 promoter activity after activation with PMA/ionomycin, which was independent of VacA concentration. A stable J-β2.7 transfectant, complemented with the human CD11a integrin gene, reexpressed functional LFA-1 on the surface (Figure S4A) and regained a concentration-dependent VacA sensitivity in the IL-2-luc assay (Figure 3B). FK506, an immunosuppressive drug which enters cells independently of a receptor, inhibited IL-2-luc activity in all cell lines (Figure 3B).

Taken together, these studies provide evidence that uptake of VacA into Jurkat T cells and the induction of acidic vacuoles and inhibition of IL-2 gene transcription is dependent on LFA-1. A residual vacuolating activity of VacA in J-β2.7 cells indicates that VacA might enter Jurkat T cells by (an) additional pathway(s), independent of CD18. Such multiple entry pathways into epithelial cell lines have been described for VacA before (Schraw et al., 2002; Ricci et al., 2000).

Reconstitution of Human β2 Integrin in CHO Cells Confers VacA Sensitivity

Our data so far suggest a functional role of LFA-1 integrins for VacA activity in lymphocytes. To study whether LFA-1 surface expression per se is sufficient for VacA uptake and which integrin heterodimers are able to confer VacA sensitivity, we used the chinese hamster ovary (CHO) cells, which obviously lack human integrins and display a very low sensitivity to VacA (Schraw et al., 2002; Ricci et al., 2000). Stably transfected CHO cells expressing human αLβ2 (CD11a/CD18, LFA-1), αMβ2 (CD11b, CD18, Mac-1), or αXβ2 (CD11c/CD18, p150, 95) transported the functional integrin heterodimers onto the surface, as determined by flow cytometry (Figure S4B) and ICAM-1 binding assays (data not shown). VacA-treated CD11/CD18-transfected CHO cells revealed a higher number of vacuolated CHO cells, as compared to control CHO cells (transfected with the pcDNA vector alone) (Figure 4A). Comparison of vacuolation by the quantitative neutral red assay supported the microscopic data (Figure 4B). Interestingly, m1-VacA-mediated cellular vacuolation correlated well with the level of CD18 surface expression of the corresponding transfected cells, irrespective of the particular CD18 heterodimers, indicating that the surface location of CD18, rather than a particular CD11 integrin subunit, determines VacA sensitivity. Stable transfection of CHO cells with human CD18 without a corresponding human CD11 counterpart resulted in CD18 surface detection by FACS analysis, but the transfectants did not gain sensitivity to VacA-mediated vacuolization (data not shown). Thus, functional human CD18 integrin heterodimers are necessary and sufficient to confer VacA sensitivity and the CD18 integrin subunit acts as a surface receptor for a highly efficient VacA binding and uptake.

Role of CD18 Integrin Intracellular Domain for VacA Sensitivity

Many functional properties of integrins depend on the integrity of their highly conserved short cytoplasmic domains (Fabbri et al., 1999, 2005). In order to clarify whether outside-in or inside-out signaling of the CD18 integrin subunit is necessary for triggering VacA-induced uptake, CHO cells stably transfected with wild-type (αLβ2; WT) or a mutant version with a complete deletion

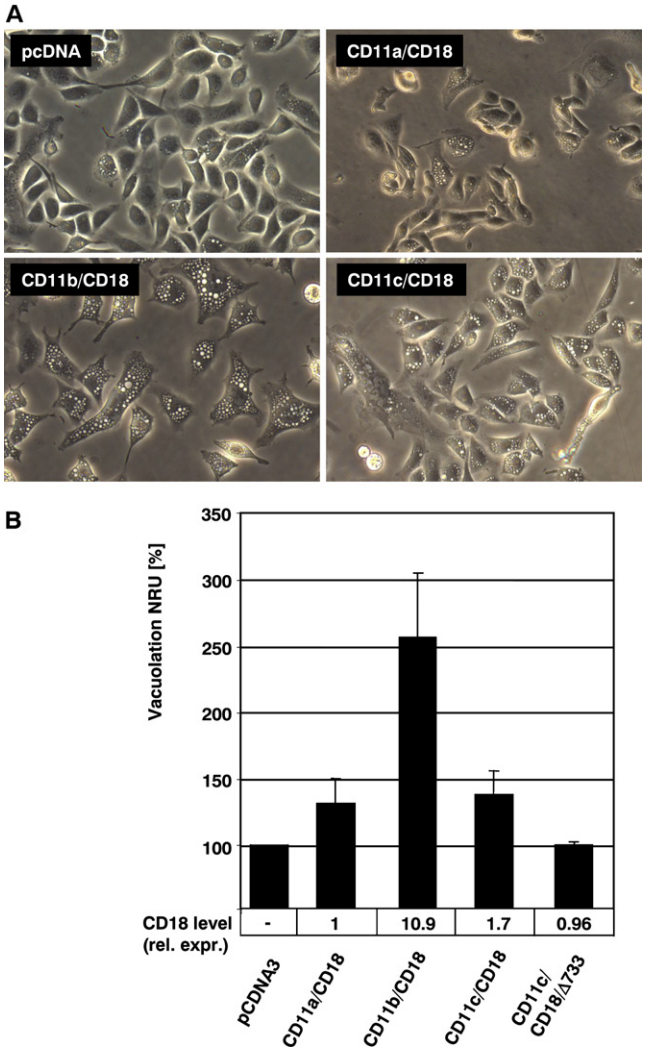

Figure 4. Expression of Human CD18 Integrin in CHO Cells Confers VacA Sensitivity

(A) Chinese hamster ovary cells (CHO) transfected with the pcDNA3 vector alone or the cloned human β2 integrin gene together with the αL (CD11a/CD18), the αM (CD11b/CD18) the αX gene (CD11c/CD18), or the β2 integrin gene with a deletion (no cytoplasmic tail) (CD11a/CD18Δ733) were cultured overnight in the presence of *Hp* P12 ccs. Vacuole formation was assessed by phase microscopy.

(B) Quantification of vacuolation by the neutral red uptake (NRU) assay. Vacuole formation in CHO cells was quantitated by monitoring the release of neutral red dye after VacA treatment. Cells were cultured in 12-well plates in triplicate overnight before the addition of 500 ng/ml purified VacA. Uptake of neutral red dye into acidic vacuoles was quantitated on a microtiter plate reader, and results were corrected for differences in cell numbers. The vacuolating activity of pcDNA3-transfected cells was set to 100%. The level of surface exposition of the integrin heterodimers (CD18-level, relative expression) was determined by FACS. Data points represent the mean ± SEM from three experiments.

of the cytoplasmic domain of CD18 (αLβ2Δ733) (Fabbri et al., 1999, 2005) were compared for VacA uptake and induction of cellular vacuolation (Figure 4B). Despite a similar surface expression level of CD11a/CD18Δ733 and WT LFA-1, the VacA-induced vacuolation of the mutant LFA-1 was as low as in the

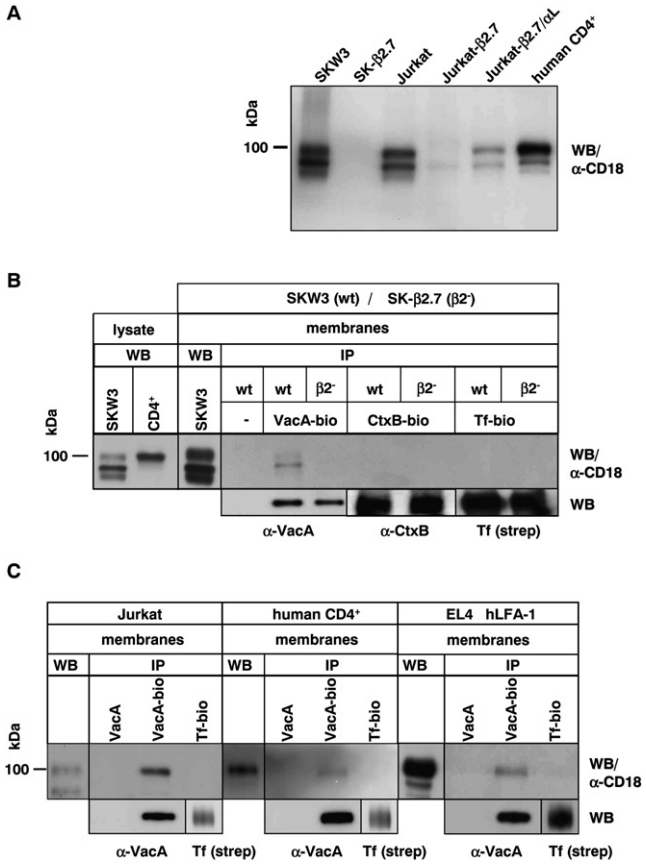

Figure 5. Immonoblots Showing CD18 Expression in Different Sets of T Cells and Immunoprecipitation to Demonstrate a Direct Interaction of m1-VacA with Human CD18 Integrin Subunit

(A) Immunoblot analysis of different human WT and CD18-deficient (SK-β2.7) or CD11a-deficient (Jurkat-β2.7) or complemented (Jurkat-β2.7/αL) T cell lines and primary human CD4+ T cells for CD18 expression using anti-CD18 antibody MEM-48.

(B) Purified membranes were prepared from SKW (WT) or β2-integrin deficient (SK-β2.7) lymphoblastoid cell lines. VacA, cholera toxin B, and transferrin were labeled with biotin (VacA-bio, CtxB-bio, and Tf-bio, respectively) and used to immunoprecipitate CD18 integrin. CtxB-bio and Tf-bio served as controls to rule out a direct binding of CD18 to biotin.

(C) Purified membranes of Jurkat, human primary CD4+, or murine EL4 T cells transfected with human LFA-1 (hLFA-1) were used for immunprecipitation, as above. Three independent experiments have been performed. WB, immunoblot; IP, immunoprecipitation; strep, streptavidin.

pcDNA-transfected control CHO cells, suggesting that intracellular signaling of the CD18 integrin subunit is essential for its VacA receptor function.

Direct Interaction of VacA and CD18

In order to demonstrate a direct binding of VacA to the CD18 subunit, VacA was biotinylated (VacA-biotin) and used in immunoprecipitation experiments. First, the CD18 production of different WT and mutant T cell lines, as well as primary human CD4+ T cells, was compared in the immunoblot (Figure 5A). Whereas the human T lymphoblastoid cell line SKW3 produces CD18, its mutant derivative SK-β2.7 is completely defective in production of CD18

(Weber et al., 1997). Jurkat-β2.7 T cells produce low amounts of CD18, which, however, does not translocate to the cell surface due to a missing CD11a integrin subunit (Figure S4A) (Weber et al., 1997). Biotinylated transferrin (Tf-biotin) or biotinylated cholera toxin B subunit (CtxB-biotin), which forms oligomers like VacA, served as controls for the immunoprecipitation. Binding and coprecipitation of CD18 with VacA-biotin was obtained with membrane preparations from SKW3, but not from SK-β2.7 cells (Figure 5B). CD18 was also precipitated from a membrane preparation of Jurkat T cells or primary human CD4$^+$ T cells using the same approach (Figure 5C). Thus, we suggest that a direct interaction of VacA and CD18 is required for VacA internalization and inhibition of IL-2 transcription.

Effect of VacA on CD4$^+$ T Cell Adhesion to ICAM-1

We next examined the impact of VacA binding to CD18 on LFA-1 function. Human CD4$^+$ T cells were treated with increasing concentrations of purified VacA (0.1–3.0 μg/ml) and their binding to recombinant human ICAM-1 was determined. Interestingly, although VacA bound to the activated T cells, it did not interfere with the interaction of activated CD4$^+$ T cells to ICAM-1 (Figure S6). In contrast, a CD18 blocking monoclonal antibody (IB4) showed a concentration-dependent inhibition of T cell binding to the ICAM-1 substrate. Thus, VacA apparently does not interfere with the binding activity of activated β2 integrin receptors to their substrate, which is in accordance with the fact that a very specific interaction of VacA takes place with the surface integrin receptor only at the uropod. This scenario suggests a defined activation state of the integrin to be recognized by VacA, consistent with a single CD18 integrin species coprecipitating with VacA (Figures 5B and 5C). As shown in the total cell lysates (Figure 5A), SKW3, Jurkat, and primary human CD4$^+$ T cells express diverse subtypes of CD18, probably representing different integrin modifications (Dahms and Hart, 1985, 1986).

Resistance of Murine T Lymphocytes to s1m1-VacA

Hp is a specific pathogen of humans, but upon adaptation, the bacteria do also colonize laboratory animals. *H. pylori vacA* null mutant strains can successfully colonize the stomachs of several animal models, including gnotobiotic piglets, mice, and Mongolian gerbils (Eaton et al., 1997; Ogura et al., 2000; Wirth et al., 1998; Marchetti et al., 1995; Salama et al., 2001). *Hp*-infected mice show a mild gastric inflammation with no typical gastroduodenal disease. We analyzed the putative interaction of VacA and its effect on IL-2 secretion on murine T cells by using the lymphoblast T cell line EL4 (ATCC TIB-39), and primary mouse spleen CD4$^+$ T cells from BALB/c and C57BL/6 mice. In contrast to human T cells, murine T cells did not significantly respond to m1-VacA treatment, neither by vacuolation (data not shown) nor by inhibition of IL-2 secretion (Figure 6A). Similar data have been reported recently for primary murine splenocytes or CD4$^+$ T cells (Algood et al., 2007).

A possible reason for this lack of activity might be the rather inefficient binding of m1-VacA to murine as compared to human T cells, as demonstrated by a quantitative flow cytometric binding assay (Figure 6K). $VacA_{Alexa}$ (green) associated to the uropod (Figures 6H and 6J), but in contrast to Alexa555-labeled cholera toxin B (CtxB) (red), VacA was never internalized by murine CD4$^+$ T cells, even after PMA activation (Figures 6C–6J).

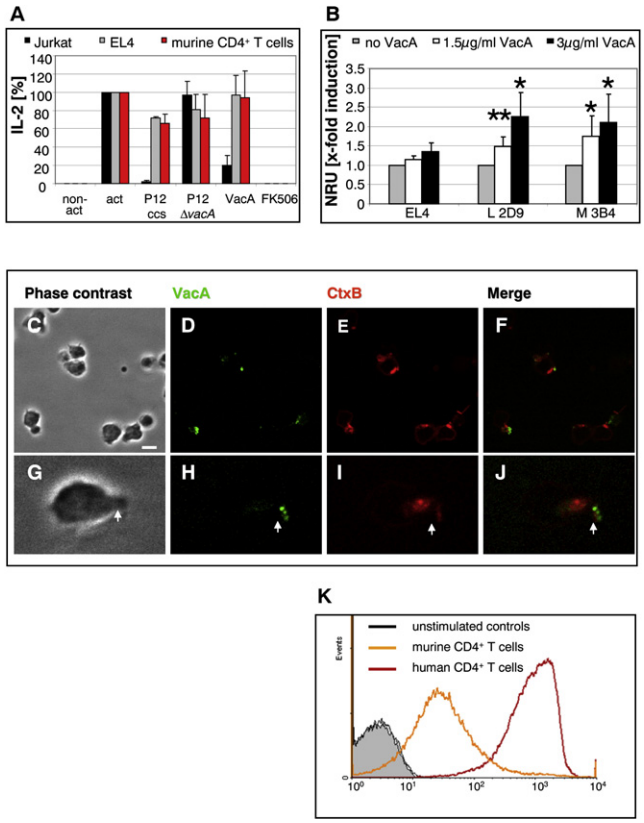

Figure 6. *Hp* m1-VacA Fails to Inhibit IL-2 Production in Murine T Cells Due to a Low Binding Activity and Failure of Cellular Internalization

(A) To measure IL-2 secretion by ELISA, Jurkat T cells, murine EL4 T cells, and primary murine CD4$^+$ T cells purified from the spleen of mice (C57BL/6) were analyzed. Cells preactivated by PMA were infected by *Hp* ccs, purified VacA, or FK506 as indicated; stimulated with PMA/ionomycin; and compared with stimulated noninfected control (100%). Data points represent the mean ± SEM from three experiments.

(B) VacA-induced cellular vacuolation in EL4 WT or LFA-1- (clone 2D9) or Mac-1 (clone 3B4)-humanized EL4 T cells was measured by the quantitative neutral red assay (NRU). Vacuolation is presented as ×-fold induction as compared to nontreated cells. n = 6, not normally distributed, Mann-Whitney Sum U test (SigmaSTAT), two-tailed. *$p < 0.05$, **$p < 0.01$. Data points represent the mean ± SEM from three experiments.

(C–J) Murine CD4$^+$ T cells activated by PMA migrating on murine ICAM-1. $CtxB_{Alexa}$ (red) was readily internalized (30 min, 37°C) (E–F, I, and J), whereas $VacA_{Alexa}$ (green) binds with low affinity to the uropod of migrating T cells (H and J) and is not internalized (0–12 hr). No colocalization of VacA and CtxB was detected on these cells. Micrographs (G)–(J) represent a higher magnification of a single CD4$^+$ T cell. Bar represents 5 μm.

(K) Quantification of $VacA_{Alexa}$ binding to unstimulated and PMA preactivated (10 nM) murine or human CD4$^+$ T cells by flow cytometry.

Humanized Murine EL4 T Cells

Murine EL4 T cells were then reconstituted with single human CD11a, CD11b, or CD18 genes, and appropriate combinations thereof. Clones were selected for stable expression and enriched for high level surface localization of the corresponding antigens by FACS sorting (Figure S7A). Purified m1-VacA induced a concentration-dependent increase in vacuolation in both LFA-1 and Mac-1-humanized EL4 T cells, as measured by the

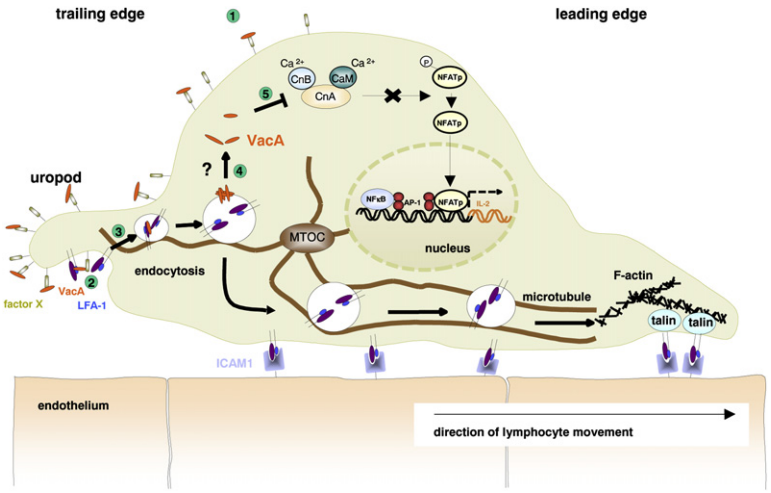

Figure 7. Working Model of the CD18-Dependent Uptake of m1-VacA into T Cells and Modulation of the T Cell Response

(1) VacA binds to a so far unknown probably GPI-anchored membrane-associated protein (factor X). (2) Upon activation of the T cell and subsequent spreading, VacA is recruited together with factor X to the uropod of the trailing edge. (2) VacA interacts with CD18 of a defined activation state. (3) The integrin heterodimer LFA-1 is internalized upon migration of the T cell (or constantly in T cell lines), and VacA inserts into the endosomal membrane forming a channel inducing cellular vacuolation. (4) In human T cells VacA is able to escape into the cytosol. (5) VacA might block calcineurin activity, which results in inhibition of IL-2 transcription and immunomodulation.

quantitative neutral red assay (Figure 6B). In contrast to vacuolation, no significant inhibition of IL-2 secretion of humanized EL4 T cells by m1-VacA was observed (Figure S7B and S7C), although a direct binding of m1-VacA to the human CD18 subunit expressed in murine EL4 cells (EL4 hLFA-1) was detected (Figure 5C).

DISCUSSION

The receptor described here, the integrin receptor CD18, is a VacA receptor specific for immune cells. Several putative receptors for VacA have been previously identified on epithelial cells (Yahiro et al., 1999, 2003), but mostly on transformed cell lines rather than on primary cells. CD18 is apparently not the sole surface molecule on T cells interacting with VacA, since in the Jurkat-β2.7 cell line VacA is still able to bind (data not shown). However, an important function of CD18 for binding and/or uptake of VacA into T cells is evident from CD18-defective Jurkat T cells, as well as from genetic complementation of murine (EL4 T cells) or hamster (CHO) cell lines. Finally, a direct interaction of VacA with human CD18 by immunoprecipitation supported the functional and microscopic VacA/CD18 colocalization data and identified CD18 as a receptor or coreceptor for VacA.

The Ca^{2+}-dependent protease calpain is required for the release of integrins from the cytoskeleton, permitting their lateral mobility in the membrane and subsequent clustering (Hogg et al., 2003; Stewart et al., 1998). Inhibition of calpain by the specific peptide inhibitor calpeptin interferes with the internalization of integrins and clearly blocked the uptake of VacA into PMA-activated $CD4^+$ T cells (Figure 1C). However, a panel of α-CD18 antibodies (IB4, MEM-48, MEM-148, and MCA503) did not interfere with VacA-mediated IL-2 inhibition in PMA-activated $CD4^+$ T cells (data not shown), indicating that they did not interfere with the binding site of VacA on CD18 or did not block the uptake or internalization of CD18. Thus, in contrast to many cell lines, uptake of VacA into primary T cells and its effects on cellular function is definitely dependent on the presence of the CD18 receptor and its cellular internalization.

Several other prominent bacterial toxins and also viruses use integrins for their internalization into host cells. Thus, CD11a/CD18 has been identified as a receptor for RTX toxins, such as the *Escherichia coli* hemolysin or the leukotoxin of the dental pathogen *Actinobacillus actinomycetemcomitans* (Lally et al., 1997). The adenylate cyclase toxin of *Bordetella pertussis* binds to target cells of the immune system via CD11b/CD18 (Guermonprez et al., 2001) and enters the cells to shape with its enzymatic activity the local adaptive immune response toward tolerance of the pathogen (Vojtova et al., 2006).

Since CD18 is usually absent on the membrane of epithelial cells, the route of uptake and intracellular trafficking of VacA and its ability to escape into the cytosol and to interfere with cytokine signaling seems to depend on its route of entry. There might be significant differences in binding of VacA and uptake between epithelial cells and immune cells (Gauthier et al., 2005), as well as between T cells of murine and human origin (see Figure S7). The unexpected functional differences between human and murine humanized T cells in inhibition of VacA-mediated IL-2 expression suggest a highly specific pathway in human T cells for VacA internalization. Thus, differences in a late step of VacA intoxication, beyond the vacuolation step, e.g., translocation of VacA from the vacuole into the cytosol, or interaction with the cytosolic VacA target might be incompatible in the mouse model. This topic is presently the subject of intensive investigations. Our data are summarized in a current model of VacA action on T lymphocytes (Figure 7). As a final consequence, the high species specificity of VacA seriously challenges the suitability of the mouse model for many *Hp* pathogenesis studies, since neither is the *cag*-T4SS stable in the mouse model (Philpott et al., 2002), nor is there an activity of m1-VacA on IL-2 secretion in mouse T cells (this work).

Finally, we have shown that m1-VacA enters human T lymphocytes via the CD18 integrin receptor. Besides its multiple activities on epithelial cells, which represent a first barrier for *Hp*, the use of CD18 as a receptor qualifies VacA as an immunomodulin. The IL-2 modulating activity of VacA, together with the recently described inhibition of T cell proliferation by *Hp gamma*-glutamyl transpeptidase (Schmees et al., 2007), impressively underscores the general immune subversion activities of *Hp* (Baldari et al., 2005). Since LFA-1 is an integrin receptor present on all leukocytes subsets, VacA might use it also in granulocytes, macrophages, dendritic cells, B cells, or NK cells. Indeed, initial experiments using $VacA_{Alexa}$ for infection of primary human dendritic

cells (DCs) show similar results as those for T cells. VacA preferentially binds to the uropod of DCs and colocalizes with CD18, followed by its strong intracellular accumulation in a perinuclear localization (data not shown). Taken up by DCs, VacA might also be transported to the local lymph node and delivered to T cells or influence a successful DC/T cell interaction. Thus, our data indicate that VacA has a great potential to immunomodulate its human host and to contribute to the chronicity of the *Hp* infection. On the other hand, these results may promote the design of VacA analogs with an innovative immunosuppressive mechanism, which may be useful in widespread pathologic conditions, such as autoimmunity and transplantation.

EXPERIMENTAL PROCEDURES

Strains and Cell Culture

Hp strain P12 and 60190 and its isogenic mutants Δ*vacA* have been described previously (Gebert et al., 2003). P12 and 60190 produce an s1m1-VacA. Purified s1m1-VacA from *H. pylori* strain 60190 has been used for all VacA experiments unless indicated differently. Jurkat E6.1 T cells (ATCC TIB-152), Jurkat-β2.7 (αL-deficient), SKW3 T cells, SK-β2.7 (β2-deficient), and murine EL4 T cells (ATCC TIB-39) were maintained in RPMI 1640 medium (GIBCO) supplemented with 10% fetal bovine serum (FCS; GIBCO). CHO cells were maintained in Nutrient Mixture F12 (HAM) medium (GIBCO) supplemented with 10% FCS. If necessary for selection, G418 (1 mg/ml) (PAA), Hygromycin (150 μg/ml) (PAA), or Zeocin (200 μg/ml) (Invitrogen) was added.

Preparation of Human PBLC and Isolation of Primary CD4$^+$ T Cells

PBLC were isolated from human peripheral blood of *Hp*-negative donors as described (Gebert et al., 2003). Briefly, Ficoll-Hypaque density gradient centrifugation using Biocoll separating solution (density 1.077 g/ml; Biochrom AG) was applied, followed by positive selection of CD4$^+$ T cells using the MACS cell separation system (Miltenyi Biotec) with beads coated with anti-human CD4 antibody. Purification was performed according to the manufacturer's instructions. Cells were maintained in RPMI medium containing 10% FCS and Gentamicin (50 μg/ml; Sigma) at 37°C in a 5% CO_2 atmosphere.

T Cell Stimulation

For IL-2 inhibition experiments, primary T cells (5 × 10^5/ml) were treated with 250 μg/ml P12 concentrated culture supernatant (ccs), P12Δ*vacA* ccs, or varying concentrations (0.025–5 μg/ml) of purified, acid-activated VacA (m1 VacA, isolated from *Hp* 60190) with or without pretreatment with 20 nM PMA for the indicated time. After 1 hour, cells were stimulated with α-CD3 (HIT3a, BD Biosciences) and α-CD28 (CD28.2, BD Biosciences), either soluble or bound to polystyrene beads for 18–22 hr. Polystyrene beads (Polysciences) were coated with anti-human CD3 and CD28 monoclonal antibodies for CD4$^+$ T cell activation. In short, 5 × 10^7 beads were resuspended in 15 ml cold PBS (GIBCO) and incubated for 1.5 hr at 37°C using 5 μg anti-CD3 and 20 μg anti-CD28. After centrifugation at 8750 g for 10 min at 4°C, beads were blocked with RPMI 10% FCS for 1 hr at room temperature. Beads were resuspended in RPMI 10% FCS to a concentration of 1 × 10^5 beads per microliter. For activation of primary lymphocytes, 1.5 beads per cell were used. IL-2 production was measured as described (Gebert et al., 2003).

Isolation of Primary Murine T Cells from Spleen

Freshly removed mouse spleens were homogenized (cell strainer, pore size 70 μm; BD Falcon) with 3 ml PBS/2 mM EDTA. Cell suspension was further homogenized by pipetting and passing through a second cell strainer (pore size 40 μm; BD Falcon). Petri dish was repeatedly washed with PBS/EDTA. After two washing steps with PBS/2 mM EDTA and centrifugation (10 min, 300 g, 20°C), cells were resuspended in 1 ml cold PBS/2 mM EDTA/0,5%BSA and CD4 positive T cells were isolated using the MACS cell separation system (Miltenyi Biotec) according to the manufacturer's instruction.

Concentrated Culture Supernatants and Purification of *Hp* VacA

Concentrated culture supernatants (ccs) of *Hp* strains were prepared as described (Gebert et al., 2003). For VacA purification, *Hp* strain 60190 was cultured in Brucella broth supplemented either with 10% FCS, or with 2 g/l of β-methyl-cyclodextrin (Sigma). VacA was purified from culture supernatant by precipitation with a 44% saturated solution of ammonium sulfate and gel filtration chromatography using a Sephacryl S300 16/60 column (Pharmacia biotech). Fractions were tested for VacA by western blot and concentrated using Amicon ultra 100 k centrifugal filter devices. Protein concentration was determined by Bradford assay. All experiments were performed with acid-activated VacA preparations. 0.2 (v/v) of 300 mM HCl was added to VacA solution, incubated for 30 min at 37°C and neutralized with the same volume of 300 mM NaOH.

IL-2 ELISA

Jurkat T cells, EL4 T cells, and freshly isolated primary human and murine T cells were preincubated with 10 nM phorbol myristate acetate (PMA; Sigma) overnight, counted in a Neubauer chamber, and seeded at a cell density of 3 to 5 × 10^5 cells/ml into 48-well plates the next day. Cells were treated with 150 μg/ml of concentrated culture supernatant (ccs) or 500 ng/ml purified VacA. Before addition to cells, purified toxin preparations were acid activated (as described above). After 1 hour of incubation (37°C, 5% CO_2), cells were stimulated with PMA (10 nM) and ionomycin (1 μM) for 18–20 hr. Cells were removed by centrifugation and the supernatant was assayed for the presence of IL-2 (BD OptEIA™ IL-2 Sets, BD Biosciences).

FACS Analysis to Determine Integrin Expression and Surface Heterodimer Formation

For flow cytometric analysis of the expression of human integrin subunits, monoclonal antibodies G43-25B (CD11a), ICRF44 (CD11b), B-ly6 (CD11c), and 6.7 (CD18) from BD Biosciences were used. For the analysis, a Coulter Epics XL-MCL flow cytometer was used and the data were processed with the WinMDI 2.8 software. Live cell experiments were performed with an UltraView LCI spinning disk confocal system (PerkinElmer Life and Analytical Sciences) fitted on a Nikon Eclipse TE300 microscope equipped with a temperature- and CO_2-controllable environment chamber.

Quantification of VacA-Induced Vacuolation

EL4 or Jurkat T cells were incubated with varying concentrations of acid-activated VacA for 6 hr. NH_4Cl was added to a final concentration of 1 mM and cells were further incubated for at least 2 hr. Vacuolation was quantified by neutral red staining (Cover et al., 1992). Therefore, cells were centrifuged, resuspended in RPMI/10%FCS with 0.008% neutral red, and incubated for 10 min. After two washing steps with PBS/0.5%BSA, neutral red was extracted with 70% Ethanol, 0.37% HCl and transferred into a 96-well plate (Corning Life Sciences). Neutral red was quantified by absorption at 534 nm (reference wavelength 405 nm) using a Tecan Sunrise microtiter plate reader.

Live Cell Imaging Microscopy

VacA was fluorescently labeled using Alexa Fluor 555/488-succinimidyl ester (Molecular Probes). Labeling reaction was performed according to the manufacturer's instructions.

Statistical Analysis

Data are presented as mean ± SEM. Differences between groups were assessed by the paired, two-tailed Student's t test or by the Mann-Whitney *U* test for unpaired groups depending on the data set of concern (see figure legends).

Supplemental Data

The Supplemental Data include Supplemental Experimental Procedures, seven supplemental figures, and five supplemental movies and can be found with this article online at http://www.cellhostandmicrobe.com/cgi/content/full/3/1/20/DC1/.

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REFERENCES

Algood, H.M., Torres, V.J., Unutmaz, D., and Cover, T.L. (2007). Resistance of primary murine CD4+ T cells to *Helicobacter pylori* vacuolating cytotoxin. Infect. Immun. *75*, 334–341.

Atherton, J.C., Cao, P., Peek, R.M., Tummuru, M.K.R., Blaser, M.J., and Cover, T.L. (1995). Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. J. Biol. Chem. *270*, 17771–17777.

Baldari, C.T., Lanzavecchia, A., and Telford, J.L. (2005). Immune subversion by *Helicobacter pylori*. Trends Immunol. *26*, 199–207.

Blaser, M.J., Perez-Perez, G.I., Kleanthous, H., Cover, T.L., Peek, R.M., Chyou, P.H., Stemmermann, G.N., and Nomura, A. (1995). Infection with *Helicobacter pylori* strains possessing *cagA* is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res. *55*, 2111–2115.

Boncristiano, M., Paccani, S.R., Barone, S., Ulivieri, C., Patrussi, L., Ilver, D., Amedei, A., D'Elios, M.M., Telford, J.L., and Baldari, C.T. (2003). The *Helicobacter pylori* Vacuolating Toxin Inhibits T Cell Activation by Two Independent Mechanisms. J. Exp. Med. *198*, 1887–1897.

Cover, T.L., and Blanke, S.R. (2005). *Helicobacter pylori* VacA, a paradigm for toxin multifunctionality. Nat. Rev. Microbiol. *3*, 320–332.

Cover, T.L., and Blaser, M.J. (1992). Purification and characterization of the vacuolating toxin from *Helicobacter pylori*. J. Biol. Chem. *267*, 10570–10575.

Cover, T.L., Hanson, P.I., and Heuser, J.E. (1997). Acid-induced dissociation of VacA, the *Helicobacter pylori* vacuolating cytotoxin, reveals its pattern of assembly. J. Cell Biol. *138*, 759–769.

Cover, T.L., Vaughn, S.G., Cao, P., and Blaser, M.J. (1992). Potentiation of *Helicobacter pylori* vacuolating toxin activity by nicotine and other weak bases. J Infect. Dis. *166*, 1073–1078.

Czajkowsky, D.M., Iwamoto, H., Cover, T.L., and Shao, Z. (1999). The vacuolating toxin from *Helicobacter pylori* forms hexameric pores in lipid bilayers at low pH. Proc. Natl. Acad. Sci. USA *96*, 2001–2006.

Dahms, N.M., and Hart, G.W. (1985). Lymphocyte function-associated antigen 1 (LFA-1) contains sulfated N-linked oligosaccharides. J. Immunol. *134*, 3978–3986.

Dahms, N.M., and Hart, G.W. (1986). Influence of quaternary structure on glycosylation. Differential subunit association affects the site-specific glycosylation of the common beta-chain from Mac-1 and LFA-1. J. Biol. Chem. *261*, 13186–13196.

Eaton, K.A., Cover, T.L., Tummuru, M.K., Blaser, M.J., and Krakowka, S. (1997). Role of vacuolating cytotoxin in gastritis due to *Helicobacter pylori* in gnotobiotic piglets. Infect. Immun. *65*, 3462–3464.

Fabbri, M., Di Meglio, S., Gagliani, M.C., Consonni, E., Molteni, R., Bender, J.R., Tacchetti, C., and Pardi, R. (2005). Dynamic partitioning into lipid rafts controls the endo-exocytic cycle of the alphaL/beta2 integrin, LFA-1, during leukocyte chemotaxis. Mol. Biol. Cell *16*, 5793–5803.

Fabbri, M., Fumagalli, L., Bossi, G., Bianchi, E., Bender, J.R., and Pardi, R. (1999). A tyrosine-based sorting signal in the beta2 integrin cytoplasmic domain mediates its recycling to the plasma membrane and is required for ligand-supported migration. EMBO J. *18*, 4915–4925.

Fischer, W., Buhrdorf, R., Gerland, E., and Haas, R. (2001). Outer membrane targeting of passenger proteins by the vacuolating cytotoxin autotransporter of *Helicobacter pylori*. Infect. Immun. *69*, 6769–6775.

Fujikawa, A., Shirasaka, D., Yamamoto, S., Ota, H., Yahiro, K., Fukada, M., Shintani, T., Wada, A., Aoyama, N., Hirayama, T., et al. (2003). Mice deficient in protein tyrosine phosphatase receptor type Z are resistant to gastric ulcer induction by VacA of *Helicobacter pylori*. Nat. Genet. *33*, 375–381.

Gauthier, N.C., Monzo, P., Kaddai, V., Doye, A., Ricci, V., and Boquet, P. (2005). *Helicobacter pylori* VacA cytotoxin: A probe for a clathrin-independent and Cdc42-dependent pinocytic pathway routed to late endosomes. Mol. Biol. Cell *16*, 4852–4866.

Gebert, B., Fischer, W., Weiss, E., Hoffmann, R., and Haas, R. (2003). *Helicobacter pylori* vacuolating cytotoxin inhibits T lymphocyte activation. Science *301*, 1099–1102.

Guermonprez, P., Khelef, N., Blouin, E., Rieu, P., Ricciardi-Castagnoli, P., Guiso, N., Ladant, D., and Leclerc, C. (2001). The adenylate cyclase toxin of *Bordetella pertussis* binds to target cells via the alpha(M)beta(2) integrin (CD11b/CD18). J. Exp. Med. *193*, 1035–1044.

Hogg, N., Laschinger, M., Giles, K., and McDowall, A. (2003). T-cell integrins: More than just sticking points. J. Cell Sci. *116*, 4695–4705.

Hogg, N., Smith, A., McDowall, A., Giles, K., Stanley, P., Laschinger, M., and Henderson, R. (2004). How T cells use LFA-1 to attach and migrate. Immunol. Lett. *92*, 51–54.

Ji, X., Frati, F., Barone, S., Pagliaccia, C., Burroni, D., Xu, G., Rappuoli, R., Reyrat, J.M., and Telford, J.L. (2002). Evolution of functional polymorphism in the gene coding for the *Helicobacter pylori* cytotoxin. FEMS Microbiol. Lett. *206*, 253–258.

Lally, E.T., Kieba, I.R., Sato, A., Green, C.L., Rosenbloom, J., Korostoff, J., Wang, J.F., Shenker, B.J., Ortlepp, S., Robinson, M.K., and Billings, P.C. (1997). RTX toxins recognize a beta2 integrin on the surface of human target cells. J. Biol. Chem. *272*, 30463–30469.

Marchetti, M., Arico, B., Burroni, D., Figura, N., Rappuoli, R., and Ghiara, P. (1995). Development of a mouse model of *Helicobacter pylori* infection that mimics human disease. Science *267*, 1655–1658.

Massari, P., Manetti, R., Burroni, D., Nuti, S., Norais, N., Rappuoli, R., and Telford, J.L. (1998). Binding of the *Helicobacter pylori* vacuolating cytotoxin to target cells. Infect. Immun. *66*, 3981–3984.

Miller, M.J., Wei, S.H., Cahalan, M.D., and Parker, I. (2003). Autonomous T cell trafficking examined in vivo with intravital two-photon microscopy. Proc. Natl. Acad. Sci. USA *100*, 2604–2609.

Odenbreit, S., Püls, J., Sedlmaier, B., Gerland, E., Fischer, W., and Haas, R. (2000). Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion. Science *287*, 1497–1500.

Ogura, K., Maeda, S., Nakao, M., Watanabe, T., Tada, M., Kyutoku, T., Yoshida, H., Shiratori, Y., and Omata, M. (2000). Virulence factors of *Helicobacter pylori* responsible for gastric diseases in mongolian gerbil. J. Exp. Med. *192*, 1601–1610.

Papini, E., Satin, B., Norais, N., de Bernard, M., Telford, J.L., Rappuoli, R., and Montecucco, C. (1998). Selective increase of the permeability of polarized epithelial cell monolayers by *Helicobacter pylori* vacuolating toxin. J. Clin. Invest. *102*, 813–820.

Philpott, D.J., Belaid, D., Troubadour, P., Thiberge, J.M., Tankovic, J., Labigne, A., and Ferrero, R.L. (2002). Reduced activation of inflammatory responses in host cells by mouse-adapted *Helicobacter pylori* isolates. Cell. Microbiol. *4*, 285–296.

Ricci, V., Galmiche, A., Doye, A., Necchi, V., Solcia, E., and Boquet, P. (2000). High cell sensitivity to *Helicobacter pylori* VacA toxin depends on a GPI-anchored protein and is not blocked by inhibition of the clathrin-mediated pathway of endocytosis. Mol. Biol. Cell *11*, 3897–3909.

Salama, N.R., Otto, G., Tompkins, L., and Falkow, S. (2001). Vacuolating cytotoxin of *Helicobacter pylori* plays a role during colonization in a mouse model of infection. Infect. Immun. *69*, 730–736.

Schmees, C., Prinz, C., Treptau, T., Rad, R., Hengst, L., Voland, P., Bauer, S., Brenner, L., Schmid, R.M., and Gerhard, M. (2007). Inhibition of T-Cell Proliferation by *Helicobacter pylori* gamma-Glutamyl Transpeptidase. Gastroenterology *132*, 1820–1833.

Schraw, W., Li, Y., McClain, M.S., van der Goot, F.G., and Cover, T.L. (2002). Association of *Helicobacter pylori* vacuolating toxin (VacA) with lipid rafts. J. Biol. Chem. *277*, 34642–34650.

Seto, K., Hayashi-Kuwabara, Y., Yoneta, T., Suda, H., and Tamaki, H. (1998). Vacuolation induced by cytotoxin from *Helicobacter pylori* is mediated by the EGF receptor in HeLa cells. FEBS Lett. *431*, 347–350.

Smith, A., Bracke, M., Leitinger, B., Porter, J.C., and Hogg, N. (2003). LFA-1-induced T cell migration on ICAM-1 involves regulation of MLCK-mediated attachment and ROCK-dependent detachment. J. Cell Sci. *116*, 3123–3133.

Stewart, M.P., McDowall, A., and Hogg, N. (1998). LFA-1-mediated adhesion is regulated by cytoskeletal restraint and by a Ca2+-dependent protease, calpain. J. Cell Biol. *140*, 699–707.

Suerbaum, S., and Michetti, P. (2002). *Helicobacter pylori* infection. N. Engl. J. Med. *347*, 1175–1186.

Sundrud, M.S., Torres, V.J., Unutmaz, D., and Cover, T.L. (2004). Inhibition of primary human T cell proliferation by *Helicobacter pylori* vacuolating toxin (VacA) is independent of VacA effects on IL-2 secretion. Proc. Natl. Acad. Sci. USA *101*, 7727–7732.

Tohyama, Y., Katagiri, K., Pardi, R., Lu, C., Springer, T.A., and Kinashi, T. (2003). The critical cytoplasmic regions of the alphaL/beta2 integrin in Rap1-induced adhesion and migration. Mol. Biol. Cell *14*, 2570–2582.

Vojtova, J., Kamanova, J., and Sebo, P. (2006). Bordetella adenylate cyclase toxin: A swift saboteur of host defense. Curr. Opin. Microbiol. *9*, 69–75.

Wang, W.C., Wang, H.J., and Kuo, C.H. (2001). Two distinctive cell binding patterns by vacuolating toxin fused with glutathione S-transferase: One high-affinity m1-specific binding and the other lower-affinity binding for variant m forms. Biochemistry *40*, 11887–11896.

Weber, K.S., York, M.R., Springer, T.A., and Klickstein, L.B. (1997). Characterization of lymphocyte function-associated antigen 1 (LFA-1)-deficient T cell lines: The alphaL and beta2 subunits are interdependent for cell surface expression. J. Immunol. *158*, 273–279.

Wirth, H.P., Beins, M.H., Yang, M., Tham, K.T., and Blaser, M.J. (1998). Experimental infection of Mongolian gerbils with wild-type and mutant *Helicobacter pylori* strains. Infect. Immun. *66*, 4856–4866.

Yahiro, K., Niidome, T., Kimura, M., Hatakeyama, T., Aoyagi, H., Kurazono, H., Imagawa, K., Wada, A., Moss, J., and Hirayama, T. (1999). Activation of *Helicobacter pylori* VacA toxin by alkaline or acid conditions increases its binding to a 250-kDa receptor protein-tyrosine phosphatase beta. J. Biol. Chem. *274*, 36693–36699.

Yahiro, K., Wada, A., Nakayama, M., Kimura, T., Ogushi, K.I., Niidome, T., Aoyagi, H., Yoshino, K.I., Yonezawa, K., Moss, J., and Hirayama, T. (2003). Protein-tyrosine Phosphatase α, RPTPα, Is a *Helicobacter pylori* VacA Receptor. J. Biol. Chem. *278*, 19183–19189.